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Sitagliptin plus granulocyte colony-stimulating factor in patients suffering from acute myocardial infarction: A double-blind, randomized placebo-controlled trial of efficacy and safety (SITAGRAMI trial)

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ABSTRACT

Objective: In animal models, G-CSF based progenitor cell mobilization combined with a DPP4 inhibitor leads to increased homing of bone marrow derived progenitor cells to the injured myocardium via the SDF1/CXCR4 axis resulting in improved ejection fraction and survival after acute myocardial infarction (AMI).
Research design and methods: After successful revascularization in AMI, 174 patients were randomized 1:1 in a multi-centre, prospective, placebo-controlled, parallel group, double blind, phase III efficacy and safety trial to treatment with G-CSF and Sitagliptin (GS) or placebo. Diabetic and non-diabetic patients were included in our trial. The primary efficacy endpoint hierarchically combined global left and right ventricular ejection fraction changes from baseline to 6 months of follow-up (Δ_{LVEF}, Δ_{RVEF}), as determined by cardiac MRI.
Results: At follow-up Δ_{LVEF} as well as Δ_{RVEF} did not differ between the GS and placebo group. Patients in the placebo group had a similar risk for a major adverse cardiac event within 12 months of follow-up as compared to patients under GS.
Conclusion: Progenitor cell therapy comprising the use of G-CSF and Sitagliptin after successfully revascularized acute myocardial infarction fails to show a beneficial effect on cardiac function and clinical events after 12 months. (EudraCT: 2007–003,941-34; ClinicalTrials.gov: NCT00650143, funding: Heinz-Nixdorf foundation).

1. Introduction

Acute myocardial infarction can lead to terminal heart failure despite today's interventional and extensive pharmacological therapies. Available treatment options focus on unloading the heart but do not provide regenerative mechanisms. Circulating cardioprotective progenitor cells (ciPCs) originating from bone marrow can be recruited to the injured myocardium via the SDF1-CXCR4 signaling [1–3]. Stromal cell-derived factor 1 (SDF1) is secreted by the ischemic myocardium and is cleaved by the dipeptidyl peptidase 4 (DPP4) [4–6]. In addition to glucose control, inhibition of DPP4, e.g. by Sitagliptin, leads to increased levels of local SDF1 and thus can mediate an enhanced progenitor cell recruitment [7,8]. Besides myocardium, SDF1 has been shown to be involved in tissue regeneration of numerous other organs [9–12]. In various pre-clinical studies we have recently demonstrated that pharmacological DPP4 inhibition after acute myocardial infarction (AMI) leads to increased cardiac recruitment of circulating progenitor cells that

Abbreviations: AMI, acute myocardial infarction; BOG, Department of Cardiology, Klinikum Bogenhausen, Munich, Germany; CI, confidence interval; ciPCs, circulating progenitor cells; CXCR4, C-X-C chemokine receptor type 4; DPP4, dipeptidyl peptidase 4; G-CSF, granulocyte colony stimulating factor; GH, Department of Internal Medicine I, Klinikum Grosshadern, Ludwig-Maximilians-University, Munich, Germany; GS, G-CSF + Sitagliptin; HR, hazard ratio; INN, Department of Cardiology, Klinikum Innenstadt, Ludwig-Maximilians-University, Munich, Germany; ITT, intention to treat; LEDV, left ventricular end-diastolic volume; LESV, left ventricular end-systolic volume; L/RVEF, left/right ventricular ejection fraction; MACE, major adverse cardiac event; MRI, magnetic resonance imaging; NSTEMI, non-ST-elevation myocardial infarction; PCI, percutaneous coronary interventions; RV, right ventricle; SDF1, stromal cell-derived factor 1; STEMI, ST-elevation myocardial infarction.

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mediated an ameliorated cardiac remodeling, enhanced myocardial function and improved survival. Increasing the number of ciPCs in the peripheral blood using granulocyte colony stimulating factor (G-CSF) further boosted this therapeutic effect [3,8,13]. Due to these results, we initiated the prospective randomized phase III SITAGRAMI trial aiming to assess whether a combined application of G-CSF and Sitagliptin (GS) is superior to placebo concerning global cardiac function 6 months after AMI. We report the prespecified efficacy and the 1-year safety analyses from the SITAGRAMI trial.

2. Methods

2.1. Study population and protocol

Patients were enrolled in the study from March 2008 to June 2013 including a 12-month follow-up. Detailed inclusion and exclusion criteria as well as the study design of the SITAGRAMI trial have been published elsewhere [14]. In short, patients aged 18 years or older were eligible for enrolment after an acute (non) ST-elevation myocardial infarction that was successfully revascularized by coronary stent implantation within 2 to 24 h after onset of angina and demonstrated substantial myocardial damage (creatine kinase >540 U/l and regional wall motion abnormality in MRI analysis) [15]. Patients presenting with non-ST-elevation MI were only included into the trial when they additionally showed a totally occluded coronary target vessel during coronary angiography. The percentage of NSTEMI patients was 11.5%, STEMI patients represented 88.5% of the study population. Written informed consent was obtained from all patients. The ethics review board at each participating center approved the protocol, and the trial was performed in accordance with the Declaration of Helsinki. The trial was investigator-initiated, randomized, placebo-controlled, double-blind, parallel-group conducted in Munich, Germany, at three sites: Klinikum Grosshadern (GH), Klinikum Innenstadt (INN), and Klinikum Bogenhausen (BOG).

At screening, all patients underwent baseline cardiac MRI 2 to 6 days after revascularization. A total of 174 eligible patients were randomly assigned to receive either GS or placebo within 8 h after the initial MRI (Fig. 1). The concealed 1:1 allocation was an internet-based randomization schedule (https://wwwapp.ibe.med.uni-muenchen.de/randoulette) stratified by gender and diabetes mellitus type II. Fixed random block size was six which was not disclosed during the trial. The random number list was prepared by an investigator with no clinical involvement in the trial. Patients, clinicians, core laboratories, trial staff (data analysts, statisticians) and an independent endpoint committee were masked to the assigned treatment arm. Blinded follow-up MRI analysis was performed using identical projections 6 months after myocardial infarction. Revascularization during follow-up was performed when restenosis or de-novo stenosis >75% (as assessed by angiographic means) was detected. In addition, patients were scheduled to undergo a last safety follow-up assessment 12 months after randomization.

2.2. Study treatment

In patients assigned to intervention, G-CSF (Lenograstim [GRANOCYTE®, Chugai Pharma], 10 μg/kg/day, i.e. 1.28 Mio IU/kg/day divided in two doses subcutaneously) was given over a period of 5 days and Sitagliptin (Januvia®, MSD Sharp&Dohme) 100 mg was administrated orally each day for 28 days. In the control group, Sodium Chloride 0.9% as well as white P-tablets of 10 mm Lichtenstein® were administered as placebo. Overencapsulated Sitagliptin and Placebo tablets as well as G-CSF and Placebo syringes looked identical.

2.3. Cardiac MRI analysis

Patients were examined on a 1.5 T whole body MR system (Magnetom Avanto, Siemens Healthcare, Erlangen, Germany). Cardiac functional imaging (cine MRI) was based on a segmented steady state free precession pulse sequence using parallel imaging (temporal resolution (TR) 45 ms, spatial resolution (SR) $1.5 \times 1.4\ mm^2$). All MRI data were analyzed in a blinded manner by independent radiologists who were not aware of treatment assignment (see MRI Supplement for details).

2.4. Flow cytometry

Cytometric analysis was performed using a flow cytometer (Beckman Coulter Epics XL). Each analysis included 20.000 events. For immunophenotyping, we used the monoclonal antibodies against CD34 (Beckman Coulter) and CD45 (Becton Dickinson) conjugated with fluorescein isothiocyanate and phycoerythrin, respectively, and their corresponding isotype controls.

2.5. Endpoints

The primary efficacy end point consists of two components: absolute changes in global left and right ventricular ejection fractions (Δ_{LVEF}, Δ_{RVEF}) between screening MRI 2 to 6 days post-PCI (MRI[Scr]) and 6 months follow-up MRI (MRI[F6]). Both components were calculated as the difference between MRI[F6] and MRI [Scr]: Δ_{EF} = MRI[F6] − MRI[Scr]. Secondary end points comprised absolute changes in global and regional myocardial function, myocardial perfusion and infarct volume, as determined by MRI[Scr] and MRI[F6]. Global functional parameters comprised end-diastolic volume (LEDV) and end-systolic volume (LESV). To explore the maximum of cardiac recovery for both ventricles after intervention, a composite efficacy endpoint CEPΔ_{EF} was specified as further key secondary efficacy endpoint which was defined as follows: For this composite assessment of left and right ventricular global systolic function, Δ_{EF} was calculated for both ventricles. If the right ventricle showed late gadolinium enhancement (LGE) at screening visit and Δ_{RVEF} exceeded Δ_{LVEF}, then Δ_{RVEF} of the right ventricle replaced Δ_{LVEF}. Regional wall motion was assessed by determining segmental systolic wall thickening in the infarct and remote segments. Myocardial perfusion was evaluated by the rate of increase in signal intensity during first pass in the infarct and remote segments.

To assess the absolute change of RVEF in patients with late enhancement of the right ventricle at screening MRI, a pre-specified subgroup analysis was performed. Furthermore, we assessed peripheral blood CD34 positive cells using flow cytometry and in stent restenosis using angiography after 6 months. Besides this, the occurrence of major adverse cardiac events (MACE) was investigated, a composite safety endpoint combining the outcome sudden cardiac death, myocardial infarction besides in-stent restenosis or de-novo stenosis requiring either coronary artery bypass graft surgery (CABG) or coronary re-intervention. Only the first event for each patient was considered for the time-to-event safety analysis.

2.6. Statistical analysis

In a first step we planned to recruit 100 patients based on the following sample size calculation: a total of 40 patients in each group will have 80% power to detect a difference in means of 3.5 assuming that the common standard deviation is 5.5 using a two group t-test with a 5% two-sided significance level and assuming a dropout rate of 20%. The assumptions for our sample size calculations were based on the results of the REPAIR-AMI trial [16]. Since uncertainty on the true standard deviation existed, a blinded sample size reestimation was planned (to be performed by an independent statistician) close to the completion of the recruitment of 100 patients [17,18]. The sample size reassessment recommended a recruitment up to a total of n = 174 patients (for details refer to the protocol in the Web Supplementary Appendix).

All analyses were performed on an intention-to-treat (ITT) basis. GS and placebo groups were compared in terms of both primary endpoint

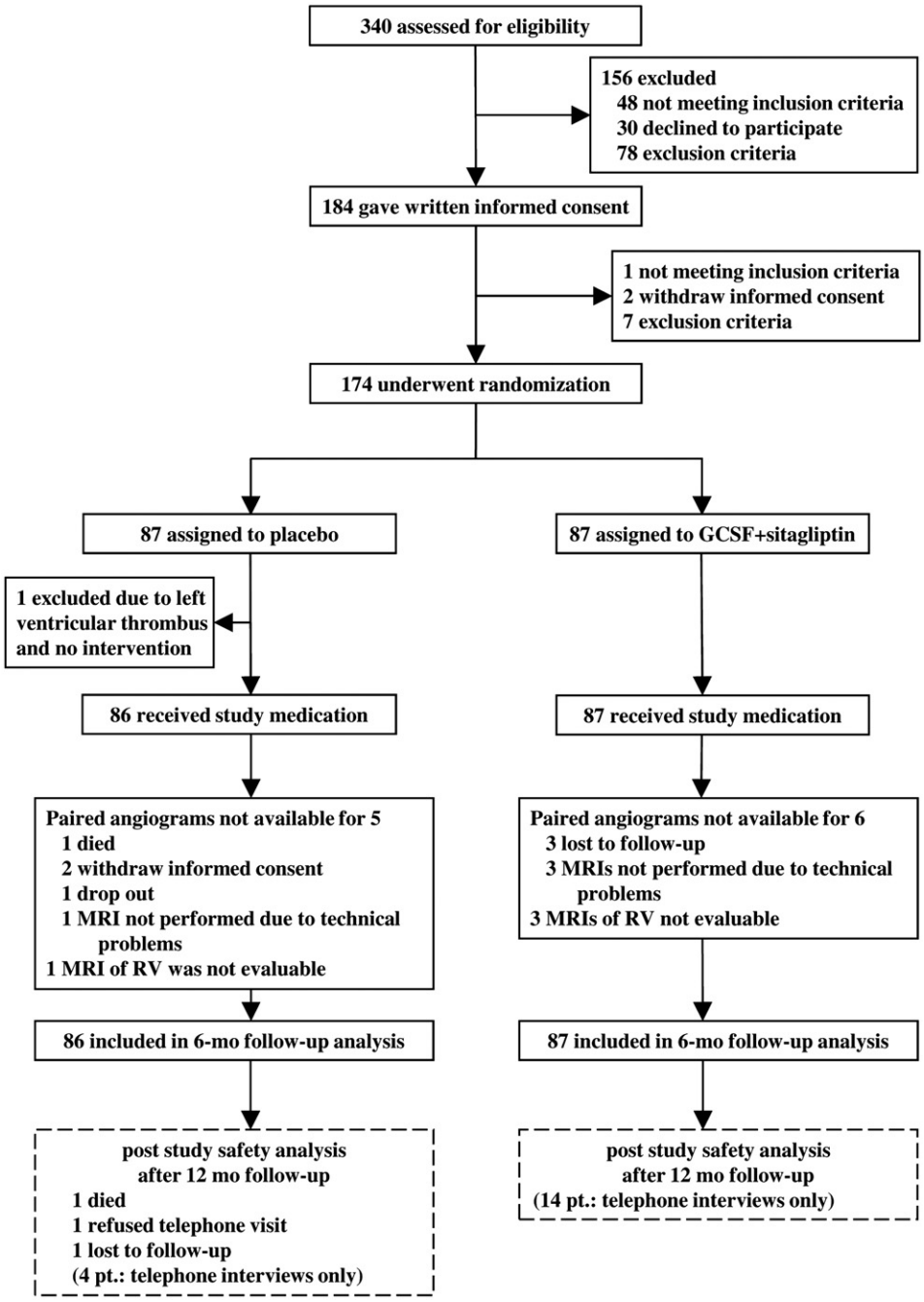

Fig. 1. Study flow chart: Enrollment and Outcomes. For the primary efficacy analyses patients with missing MRI data were handled by multiple imputation methods to comply with ITT principles.

components by an analysis of covariance (ANCOVA) with factor for treatment and baseline EF as covariate. Hierarchical testing was used to secure the overall level 1 error to 5%.

MACE-free survival was analyzed using the Kaplan–Meier method (for which patients were censored at the time of withdrawal from the study or at last follow-up), and compared between groups by means of the log-rank test. We calculated hazard ratios (HRs) and 95% CIs from a Cox regression model that was unadjusted for other covariates.

All statistical tests were two-sided; the level of significance was chosen to be 0.05.

Study database was stored in SAS (Unix Version 9.2, SAS Institute Inc., Cary, NC). Statistical analyses were performed using the statistical software package R version 3.0.1 [19], the R package "mice" was applied for multiple imputation techniques.

Further details concerning the statistical methods applied are provided in the Supplementary Appendix, available with the full text of this article.

3. Results

3.1. Enrollment and baseline characteristics

We screened a total of 340 patients with AMI and successful revascularization in terms of successful coronary stent implantation (Fig. 1). 48 did not meet inclusion criteria, 78 showed exclusion criteria and 30 refused to participate in our study. 184 patients gave written informed consent, 10 were screening failures. Finally, 174 participants were randomized (87 patients in each group). 157 patients were recruited at

the trial site GH, 10 at INN, and seven at BOG. One patient of the placebo group did not receive any study medication since a left ventricular thrombus was noticed immediately after randomization. Both groups were well balanced concerning baseline characteristics like age, sex, risk factors and concomitant medical therapy during the trial (Table 1). The GS group showed significant mobilization of CD34+ progenitor cells in peripheral blood after 5 days (58.0 cells/μl [SD 89.4 cells/μl]) in comparison to placebo (8.6 cells/μl [SD 13.9 cells/μl], p = 0.011).

3.2. Effects on global cardiac function

Left and right ventricular function and volumes were not different at baseline between the two groups (Table 2). Paired MRI analysis was not available in 6 patients in the GS group (3 lost to follow-up, 3 refused second MRI) and in 5 placebo patients (1 death, 1 drop-out, 2 withdrawals of informed consent, 1 technical problem with MRI). Concerning the right ventricle, one MRI in the placebo group, and 3 in the GS group were not evaluable due to technical reasons.

LVEF increased from 52.2% (SD 10.4%) to 56.9% (SD 11.1%) in the placebo group, and from 51.7% (SD 10.5%) to 56.2% (SD 11.2%) in the GS group. In the final ITT analysis, there is no evidence that mean Δ_{LVEF} is larger in the GS group compared to the placebo group (−0.846%; 95% CI, −3.160 to 1.468; p = 0.471). The magnitude of Δ_{LVEF} in both treatment groups was negatively related to the LVEF measured at screening MRI (−0.186%; 95% CI, −0.299 to −0.072; p = 0.002). However, also patients with an extensive reduction in LVEF (below 40% at screening) did not show a benefit from GS treatment compared to placebo (Fig. 2A and Supplement Figure S2).

RVEF remained almost unchanged from baseline to 6-month assessment in the placebo (55.0% [SD 8.8%] to 55.3% [SD 8.4%]) and GS group respectively (56.7% [SD 8.0%] to 56.8% [SD 7.4%]). For the ITT set, no significant difference between both treatment groups could be detected (Fig. 2A). The treatment effect for absolute mean Δ_{RVEF} was 0.298% (95% CI, −1.315 to 1.910; p = 0.716).

Table 1
Characteristics of the intention-to-treat population.[a]

Characteristic	Placebo (N = 86)	GS (N = 87)	p Value
Risk factor			
Age — yr	60.1 ± 11.5	61.3 ± 11.0	0.474
Male sex — no. (%)	70 (81.4)	69 (79.3)	0.878
Arterial hypertension — no. (%)	62 (72.1)	66 (75.9)	0.695
Hypercholesterolemia — no. (%)	48 (55.8)	44 (50.6)	0.591
Diabetes — no. (%)	14 (16.3)	9 (10.3)	0.272
Smoking (current or former) — no. (%)	51 (59.3)	58 (66.7)	0.398
Familiy history of MI — no. (%)	33 (38.4)	28 (32.1)	0.488
Infarct treatment			
Infarct related vessel — no. (%)			0.932
Left anterior descending coronary artery	40 (46.5)	38 (43.7)	
Left circumflex artery	30 (34.9)	32 (36.8)	
Right coronary artery	16 (18.6)	17 (19.5)	
Time from angina to PCI (hrs)	6.74 ± 5.69	6.54 ± 5.79	0.743
Drug eluting stent — no. (%)	68 (79.1)	60 (69.0)	0.180
Bare metal stent — no. (%)	17 (19.8)	25 (28.7)	0.231
Peak creatine kinase (U/l)	3080 ± 2120	3095 ± 2113	0.688
NT-pro-BNP at inclusion	1464 ± 1273	1415 ± 1321	0.703
NT-pro-BNP after 6 months	617 ± 1611	492 ± 551	0.458
Progenitor cell population[b]			
CD34 + CD45- cells (/μl blood) at baseline	8.7 ± 13.7	7.1 ± 13.7	0.282
CD34 + CD45- cells (/μl blood) at day 5	8.6 ± 13.9	58.0 ± 89.4	0.011
Medication at discharge — no. (%)			
Aspirin	86 (100)	87 (100)	1
Clopidogrel	54 (62.8)	52 (59.8)	0.801
Prasugrel	14 (16.3)	19 (21.8)	0.461
Ticagrelor	18(20.9)	16 (18.4)	0.819
Statins	86 (100)	87 (100)	1
ACE-inhibitors	79 (91.9)	74 (85.1)	0.245
Betablockers	85 (98.8)	86 (98.9)	1
Aldosterone antagonist	10 (11.6)	12 (13.8)	0.842
AT1-blocker	5 (5.8)	11 (12.6)	0.198

PCI denotes percutaneous coronary intervention, NT-pro-BNP N-terminal pro natriuretic peptide, ACE angiotensin converting enzyme.

[a] Means ± standard deviation are depicted for quantitative, absolute numbers and proportions for categorical variables. To compare continuous variables within both treatment groups, t-test or nonparametric Mann–Whitney U-test was applied. Categorical variables were compared with the Chi^2 test or Fisher's exact test, as appropriate.

[b] Progenitor cell population was assessed only for patients recruited at site GH (N = 156; Placebo: N = 78 (one missing), GS: N = 77).

3.3. Impact on further cardiac parameters

Other secondary efficacy endpoints like absolute change in composite left and right ventricular ejection fraction CEPΔ_{EF} (placebo 5.7% [SD 7.4%] vs. GS 4.7% [SD 7.3%], p = 0.362), left-ventricular end-diastolic volume (4.0 ml [SD 28.6 ml] vs. −0.8 ml [SD 30.2 ml], p = 0.298), left-ventricular end-systolic volume (−3.8 ml [SD 19.5 ml] vs. −4.7 ml [SD 19.4 ml], p = 0.751) and infarct volume (−16.2 ml [SD 19.8 ml] vs. −13.8 ml [SD 15.8 ml], p = 0.412) as well as segmental systolic wall thickening (10.0% [SD 38.1%] vs. 10.8% [SD 41.7%], p = 0.901) revealed no significant differences between both treatment groups (Table 2). The presence and transmural extent of myocardial infarction was determined by late gadolinium enhancement (<5 segments with 76–100% affection) and showed no differences between both groups at screening (50.0% vs. 51.2%, p = 0.144) and after 6 months (30.9% vs. 26.3%, p = 0.772). Likewise, myocardial perfusion did not differ between GS and placebo group, both at screening (0.73% [SD 0.67%] vs. 0.71% [SD 0.28%], p = 0.674) and after 6 months of follow-up (0.82% [SD 0.24%] vs. 0.82% [SD 0.21%], p = 0.972). In analogy, microvascular obstruction (presence of no reflow region) was not different between the treatment groups. Corresponding 95% CIs are displayed in Table 2.

3.4. Exploratory adjusted efficacy analyses

In order to explore whether estimated treatment effect varies between several pre-randomization covariates, we performed adjusted analyses for the primary efficacy outcome measures absolute change in LVEF and RVEF. Adjusting for age, sex, diabetes mellitus, maximum creatine kinase and infarct related vessel, confirmed the robustness of the overall negative result of the prespecified primary efficacy analyses, i.e. no evidence for a positive treatment effect concerning an increase in LVEF was found (Fig. 2B).

3.5. Clinical safety events

The intervention did not change the risk for MACE for up to 12 months of follow-up after randomization (HR = 0.785; 95% CI, 0.414 to 1.488; p = 0.458) in the ITT population (Fig. 3).

In total, 39 MACEs (placebo: 22 (1 patient had 2 MACEs); GS: 17) were observed until the end of the planned follow-up after 12 months. In the placebo group, two patients experienced sudden cardiac death 2 days and 7 months after myocardial infarction respectively, whereas no cases of death occurred in the GS group. Furthermore, only one severe myocardial infarction occurred in the GS group which could be successfully treated (a causal relationship was assessed as unlikely). 17 (GS: 16) revascularizations were detected. In detail, 8 patients of the placebo group (GS: 9) showed in-stent restenosis, 15 (7) de-novo stenosis, 4 (0) both of them, and 0 (1) underwent bypass surgery due to angina pectoris.

According to the study protocol, a facultative coronary reangiography was scheduled 6 months (±4 weeks) after the initial myocardial infarction. The occurrence of MACEs was mainly driven by the angiographic detection of coronary stenoses (in-stent restenosis or de-novo stenosis) during this follow-up visit. This explains the rather

Table 2
MRI measurements (baseline and 6 months) in the ITT population.

Variable[a]	Placebo (N = 86)	GS (N = 87)	p Value
Global LVEF (%)			
Baseline mean (95% CI)	52.22 (50.00, 54.44)	51.68 (49.44, 53.92)	0.734
6 Mo mean	56.93 (54.47, 59.39)	56.24 (53.77, 58.71)	0.695
Absolute difference Δ_{LVEF} mean	4.56 (2.81, 6.30)	3.66 (1.93, 5.39)	0.470
Global RVEF (%)			
Baseline mean	55.05 (53.16, 56.94)	56.68 (54.94, 58.43)	0.208
6 Mo mean	55.30 (53.44, 57.16)	56.78 (55.12, 58.44)	0.239
Absolute difference Δ_{RVEF} mean	0.55 (−0.76, 1.87)	0.44 (−0.82, 1.70)	0.903
CEPΔ_{EF} (%)[b]			
Absolute change			
Mean	5.72 (4.09, 7.35)	4.67 (3.06, 6.28)	0.362
Late gadolinium enhancement — no. (%)	40 (46.51%)	37 (42.53%)	0.812
RVEF for patients with late enhancement at screening MRI (%)[d]			
Baseline mean	53.92 (50.91, 56.93)	57.08 (54.22, 59.94)	0.128
6 Mo Mean	55.30 (52.17, 58.42)	57.64 (55.05, 60.23)	0.245
Absolute difference mean	1.65 (−0.46, 3.76)	0.44 (−1.93, 2.81)	0.440
EDV of the left ventricle (ml/m^2 body surface area)			
Baseline mean	72.15 (68.24, 76.06)	72.46 (69.39, 75.54)	0.901
6 mo mean	74.61 (70.63, 78.59)	72.34 (68.92, 75.75)	0.389
Absolute difference mean	2.58 (−0.73, 5.89)	−0.10 (−3.67, 3.46)	0.275
ESV of the left ventricle (ml/m^2 body surface area)			
Baseline mean	34.83 (31.96, 37.69)	35.42 (32.72, 38.11)	0.765
6 Mo mean	32.97 (29.70, 36.24)	32.55 (29.61, 35.49)	0.849
Absolute difference mean	−1.69 (−3.87, 0.49)	−2.40 (−4.57, −0.23)	0.645
Infarct volume (ml)			
Baseline mean	43.18 (37.02, 49.33)	40.06 (34.68, 45.44)	0.449
6 Mo mean	27.50 (23.19, 31.81)	24.76 (20.86, 28.65)	0.349
Absolute difference mean	−16.15 (−20.57, −11.72)	−13.80 (−17.37, −10.23)	0.412
Segmental systolic wall thickening (%)			
Baseline mean	17.50 (12.09, 22.90)	20.08 (14.00, 26.15)	0.529
6 Mo mean	29.00 (22.68, 35.33)	31.91 (24.39, 39.42)	0.557
Absolute difference mean	9.96 (1.43, 18.48)	10.75 (1.35, 20.15)	0.901
Late Enhancement (≥ 5 segments with 76–100% affection)			
Baseline			
No. of patients (no. missings)	42 (2)	44 (1)	
% (95% CI)	48.84 (37.90, 59.86)	50.57 (39.64, 61.47)	0.144
6 Mo			
No. of patients (no. missings)	25 (5)	21 (7)	
% (95% CI)	29.07 (19.78, 39.86)	24.14 (15.60, 34.50)	0.772
Odds ratio (95% CI)[c]	0.17 (0.05, 0.57)	0.26(0.11, 0.64)	
p-Value	0.001	0.002	
Myocardial perfusion (%)			
Baseline mean	0.73 (0.67, 0.79)	0.71 (0.65, 0.77)	0.674
6 Mo mean	0.82 (0.77, 0.88)	0.82 (0.78, 0.87)	0.972
Absolute difference mean	0.10(0.02, 0.18)	0.12 (0.04, 0.19)	0.758
Presence of no reflow region (%)			
Baseline			
No. of patients (no. missings)	49 (2)	44 (2)	
% (95% CI)	56.98 (0.45.85, 67.61)	50.57 (39.64, 61.47)	0.482
6 Mo			
No. of patients (no. missings)	0 (5)	2 (8)	
% (95% CI)	0 (0.00, 4.20)	2.30(0.28, 8.06)	0.242

[a] Means (with 95% CI) are depicted for quantitative, absolute numbers and proportions (with Clopper–Pearson 95% CI) for categorical variables. P-values were derived by Student t-test or nonparametric Wilcoxon Mann–Whitney U-test for quantitative, Chi^2- or Fisher's exact test for categorical quantities.
[b] Pre-specified composite efficacy endpoint evaluating the maximum change of EF for patients with late gadolinium enhancement at baseline (replacement of Δ_{LVEF} with Δ_{RVEF} was done for 14 patients in the Placebo, and 14 patients in the GS group).
[c] Odds ratio (with 95% CI) was calculated from paired observations within treatment groups to explore time effects for each treatment.
[d] Pre-specified subgroup analysis according to the protocol.

abrupt drop of the event-free survival curves at 6 months of follow-up (Fig. 3).

4. Discussion

The SITRAGRAMI trial shows a lack of relevant clinical benefit in cardiac function after six months for patients with acute myocardial infarction by a combined progenitor cell therapy comprising the use of GCSF and Sitagliptin. The power of the trial makes it evident that the treatment's effect, which is below 2% (upper bound of its 95% CI), in changing Δ_{LVEF} consistently resulted in no differences in Δ_{LVEF} after adjusting for age, gender, diabetes mellitus, maximal creatine kinase and infarct related vessel.

Furthermore, the data gave no evidence for a positive effect on secondary efficacy endpoints like regional myocardial contraction, infarct volumes and perfusion. In an unplanned subgroup analysis, female patients in the GS group had a significant increase in global RVEF compared to the placebo group, which we cannot explain from a clinical point of view. Further research will be necessary to investigate whether this difference is more than a random statistical effect. Regarding safety 12 months after infarction, no evidence for a difference in the risk for MACEs could be derived, indicating that our proposed experimental

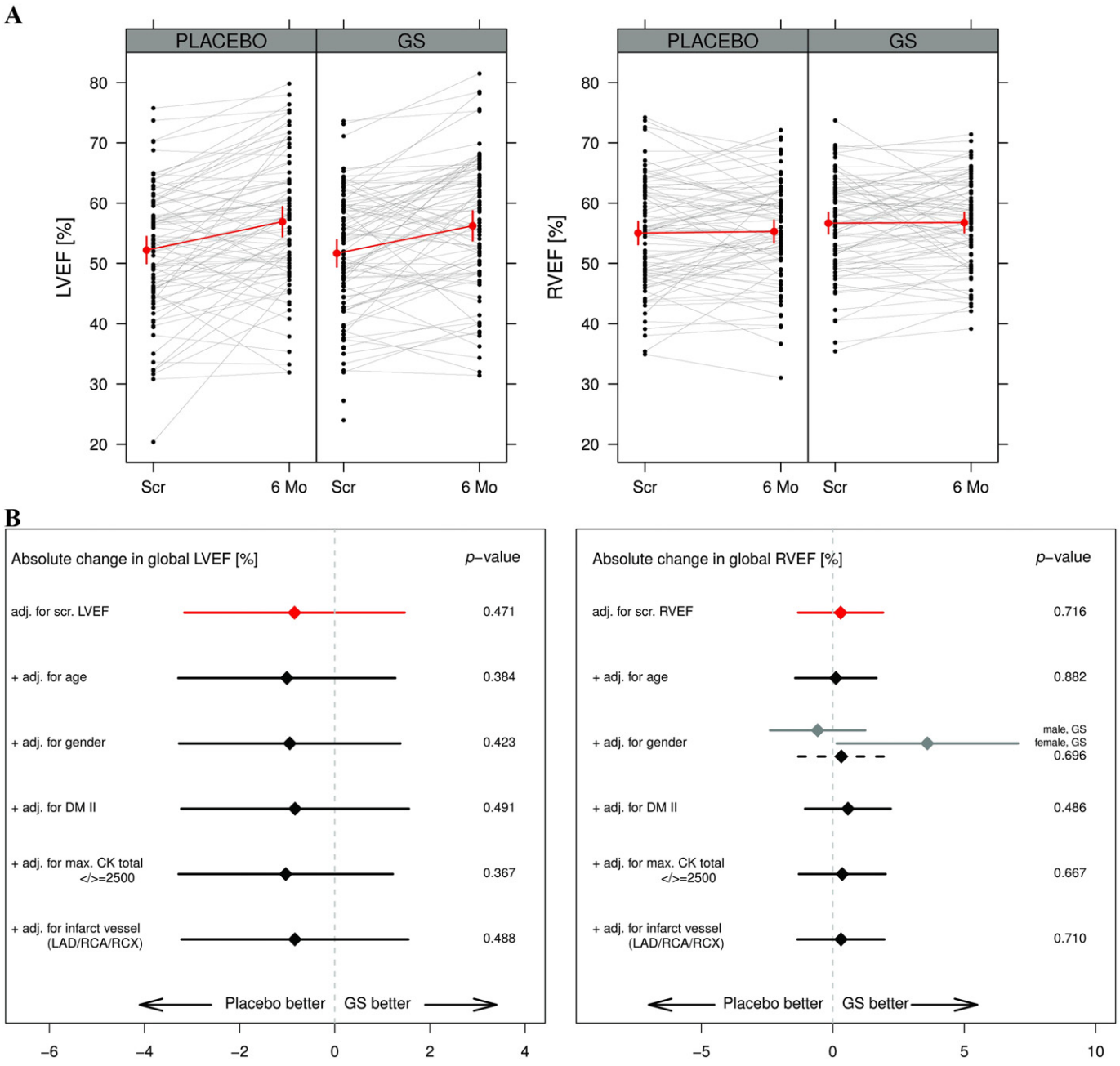

Fig. 2. (A) Trajectory plot for primary efficacy endpoints LVEF (left) and RVEF (right). For each patient a gray line indicates global ejection fraction at screening and 6 months' follow-up. Large red dots show estimated mean values, vertical bars 95% CIs. (B) Forest plots for LVEF and RVEF respectively. The treatment effect of GS therapy compared to placebo on the absolute change of global left and right ventricular EF from screening to 6 months follow-up. The estimated treatment effect is displayed before and after adjusting for additional pre-treatment covariates using analysis of covariance (ANCOVA). Red bar at the top: pre-specified confirmatory primary efficacy analysis; black bars: ANCOVA with an additional covariate as main effect. The position of the diamonds represents the point estimates of the treatment effect, i.e. the absolute change of LVEF and RVEF, respectively; the horizontal lines represent the 95% CIs. Adjusting for gender revealed that there is a significant interaction between gender and treatment group (p-value = 0.037); dashed black bar: corresponding ANCOVA model without interaction term.

therapy is safe which is in analogy to recent studies showing safety of DPP4 inhibitors concerning cardiovascular events [20,21].

We began our journey some years ago in order to advance the concept of stem cell mobilization: Since 2006, several clinical trials using only GCSF-based progenitor cell mobilization after myocardial infarction did not show a beneficial effect on the recovery of LV function. The studies ranged from our own GCSF-in STEMI trial to others, e.g. from Zohlnhöfer et al., and were confirmed by a meta-analysis of GCSF studies [22–25]. These study results stand in strong contrast to many animal studies that showed promising results after sole GCSF administration [26,27]. Thus, we focused on improving the cardiac recruitment of mobilized progenitor cells by stabilizing the essential cardiac homing factor SDF-1 through DPP4 inhibition [3,13]. The combined strategy of GCSF application and DPP4 inhibition was a novel concept that led to a striking increase of cardiac function and survival in the mouse model, which exceeded the effects observed after sole GCSF administration. This was the motivation to initiate our SITAGRAMI trial.

But how can we explain the neutral results? First, there are notable differences between the mouse model and human patients: In the mouse model, the effect of cardiac progenitor cell homing was strictly dependant on the degree of DPP4 inhibition. Though dosage was optimized for a comparable reduction of DPP4 activity to about 20% in the serum of mice and men, the needed amount of active substance fairly differed between the two species (500 mg/kg in mice and 1–2 mg/kg

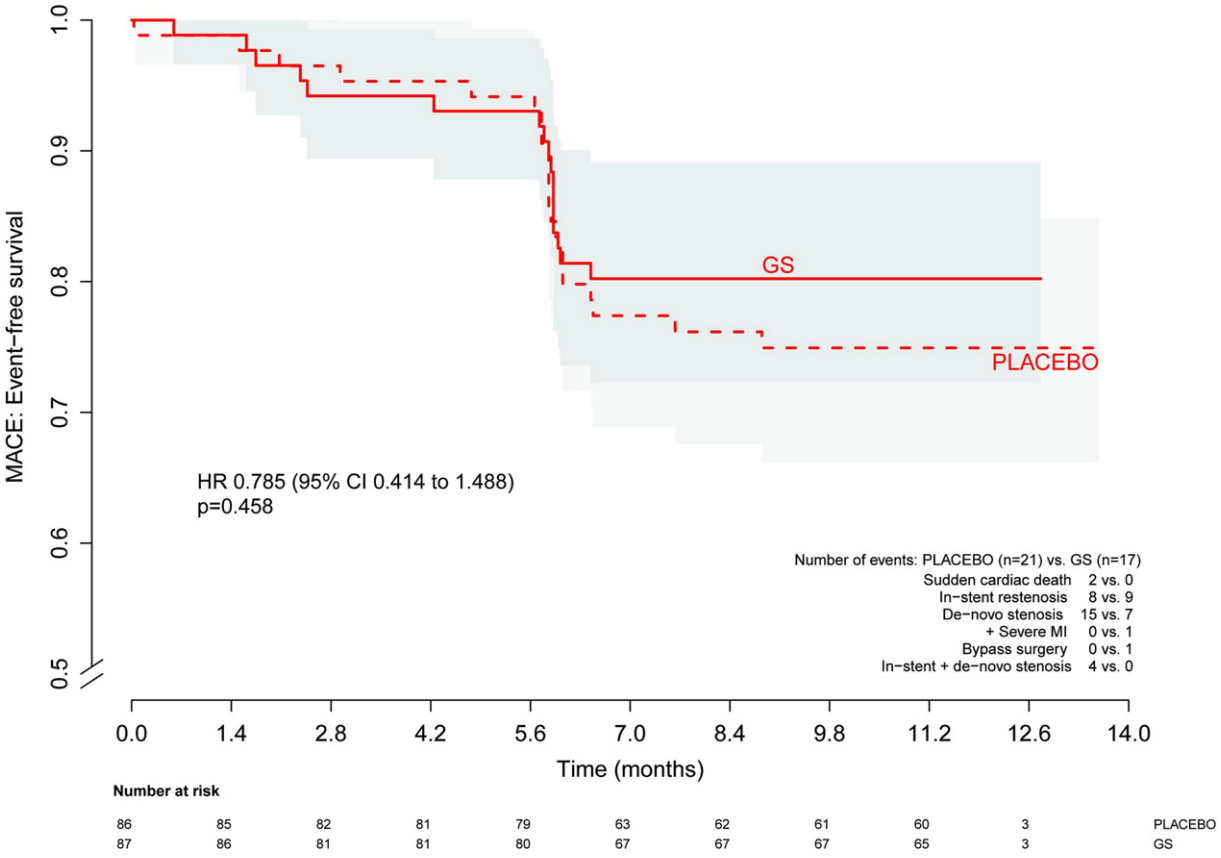

Fig. 3. Time-to-event curves for composite major adverse cardiac event (MACE) for patients in GS and placebo group. Time from randomisation to first diagnosed MACE or until censoring (time of withdrawal from the study or at last follow-up; individual planned follow-up visit approximately after 12 months). Only one patient had 2 MACE (1 occurred within the 6 months period, the second MACE before the 12 months visit). Hazard Ratio (HR) compares the risk of MACE in the experimental versus placebo group. Gray bands: 95% confidence bands using standard errors at each failure time.

body weight in humans) [8,28]. Thus, the high numerical difference and variabilities of pharmacokinetics and pharmacodynamics may have led to an insufficient effect on heart function and mortality in the patients. Second, when we were screening for myocardial recovery in our mouse model after myocardial infarction, we did not perform revascularization to maximize a potential GS-based treatment effect. Furthermore, we refrained from applying standard heart failure therapy like ACE-inhibitors or beta-blockers for the same reason. Hence, the therapeutic effect of GS therapy may have been more pronounced in our animal model making it more difficult to achieve a relevant Sitagliptin/GCSF-based add-on effect on myocardial recovery in humans. Third, cardiac function of our study patients was largely maintained and showed a mean initial ejection fraction of the left ventricle of 52% in comparison to 47% in the REPAIR-AMI trial [16]. Mainly due to presently available quick interventional and extensive standard pharmacological heart failure therapies, the average ejection fraction of patients suffering from STEMI for the first time only in a minority of cases drops below 55% [29]. Thus, we were able to recruit only a limited number of patients with a low EF that was based on an acute myocardial injury rather than chronic ischemic cardiomyopathy. However, the intervention with GCSF and Sitagliptin was consistently deemed to be not beneficial for patients with LVEF below 40 or 50%. Fourth, timing of study drug administration might be an issue. Although we initiated study drug administration at the earliest time point after cessation of post-MI stunning and acquisition of the baseline cardiac MRI we may have missed the very early phase of myocardial remodeling.

There are two major limitations of the SITAGRAMI trial: 90% of the patients were recruited at only one site though it was designed as a multi-centre study. This could have had a potential influence on patient recruitment and guidance throughout the trial. Furthermore, only 14% of our patients had a LVEF below 40% (see Supplement) (21% below 50%), thus we cannot make reliable statements on the subgroup with extensive reduction of cardiac function.

In summary, the SITAGRAMI trial revealed an overall neutral result and there was no evidence that a combined GCSF and Sitagliptin administration after AMI improves myocardial function by a clinically relevant amount in our study population. We also did not find evidence for a changed risk profile (occurrence of death, infarction and re-intervention rate) under GS therapy. However, hopeful tools for cardiac regeneration may lie in reprogramming, purification and culture of cardiac progenitor cells in the future.

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Conflicts of interest

The Ludwig-Maximilians-University owns the patents “Use of G-CSF for Treating Ischemia” (EP 03 02 4526.0 and US 60/514.474) and Remedies for Ischemia“(EP2007/003,272 and US 60/792,943). WMF and CB received honorarium from MSD for scientific presentations and advisory board participation, respectively.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijcard.2015.11.180.

References

[1] J.M. Hill, G. Zalos, J.P. Halcox, et al., Circulating endothelial progenitor cells, vascular function, and cardiovascular risk, N. Engl. J. Med. 348 (2003) 593–600.
[2] A.A. Kocher, M.D. Schuster, M.J. Szabolcs, et al., Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function, Nat. Med. 7 (2001) 430–436.
[3] M.M. Zaruba, H.D. Theiss, M. Vallaster, et al., Synergy between CD26/DPP-IV inhibition and G-CSF improves cardiac function after acute myocardial infarction, Cell Stem Cell 4 (2009) 313–323.
[4] A.T. Askari, S. Unzek, Z.B. Popovic, et al., Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy, Lancet 362 (2003) 697–703.
[5] D.J. Ceradini, A.R. Kulkarni, M.J. Callaghan, et al., Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1, Nat. Med. 10 (2004) 858–864.
[6] K.W. Christopherson II, G. Hangoc, C.R. Mantel, H.E. Broxmeyer, Modulation of hematopoietic stem cell homing and engraftment by CD26, Science 305 (2004) 1000–1003.
[7] D.J. Drucker, M.A. Nauck, The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes, Lancet 368 (2006) 1696–1705.
[8] H.D. Theiss, L. Gross, M. Vallaster, et al., Antidiabetic gliptins in combination with G-CSF enhances myocardial function and survival after acute myocardial infarction, Int. J. Cardiol. 168 (2013) 3359–3369.
[9] C. Brenner, N. Krankel, S. Kuhlenthal, et al., Short-term inhibition of DPP-4 enhances endothelial regeneration after acute arterial injury via enhanced recruitment of circulating progenitor cells, Int. J. Cardiol. 177 (2014) 266–275.
[10] S. Rafii, Z. Cao, R. Lis, et al., Platelet-derived SDF-1 primes the pulmonary capillary vascular niche to drive lung alveolar regeneration, Nat. Cell Biol. 17 (2015) 123–136.
[11] M.S. Penn, SDF-1:CXCR4 axis is fundamental for tissue preservation and repair, Am. J. Pathol. 177 (2010) 2166–2168.
[12] L. Salcedo, N. Sopko, H.H. Jiang, M. Damaser, M. Penn, M. Zutshi, Chemokine upregulation in response to anal sphincter and pudendal nerve injury: potential signals for stem cell homing, Int. J. Color. Dis. 26 (2011) 1577–1581.
[13] H.D. Theiss, M. Vallaster, C. Rischpler, et al., Dual stem cell therapy after myocardial infarction acts specifically by enhanced homing via the SDF-1/CXCR4 axis, Stem Cell Res. 7 (2011) 244–255.
[14] H.D. Theiss, C. Brenner, M.G. Engelmann, et al., Safety and efficacy of SITAgliptin plus GRanulocyte-colony-stimulating factor in patients suffering from acute myocardial infarction (SITAGRAMI-trial)—rationale, design and first interim analysis, Int. J. Cardiol. 145 (2010) 282–284.
[15] K. Thygesen, J.S. Alpert, H.D. White, Universal definition of myocardial infarction, J. Am. Coll. Cardiol. 50 (2007) 2173–2195.
[16] V. Schachinger, S. Erbs, A. Elsasser, et al., Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction, N. Engl. J. Med. 355 (2006) 1210–1221.
[17] T. Friede, M. Kieser, Blinded sample size recalculation for clinical trials with normal data and baseline adjusted analysis, Pharm. Stat. 10 (2011) 8–13.
[18] M. Kieser, T. Friede, Simple procedures for blinded sample size adjustment that do not affect the type I error rate, Stat. Med. 22 (2003) 3571–3581.
[19] R Core Team, A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2013, http://www.R-project.org.
[20] W.B. White, C.P. Cannon, S.R. Heller, et al., Alogliptin after acute coronary syndrome in patients with type 2 diabetes, N. Engl. J. Med. 369 (2013) 1327–1335.
[21] J.B. Green, M.A. Bethel, P.W. Armstrong, et al., Effect of sitagliptin on cardiovascular outcomes in type 2 diabetes, N. Engl. J. Med. 373 (2015) 232–242.
[22] M.G. Engelmann, H.D. Theiss, C. Hennig-Theiss, et al., Autologous bone marrow stem cell mobilization induced by granulocyte colony-stimulating factor after subacute ST-segment elevation myocardial infarction undergoing late revascularization: final results from the G-CSF-STEMI (granulocyte colony-stimulating factor ST-segment elevation myocardial infarction) trial, J. Am. Coll. Cardiol. 48 (2006) 1712–1721.
[23] D. Zohlnhofer, A. Dibra, T. Koppara, et al., Stem cell mobilization by granulocyte colony-stimulating factor for myocardial recovery after acute myocardial infarction: a meta-analysis, J. Am. Coll. Cardiol. 51 (2008) 1429–1437.
[24] D. Zohlnhofer, I. Ott, J. Mehilli, et al., Stem cell mobilization by granulocyte colony-stimulating factor in patients with acute myocardial infarction: a randomized controlled trial, JAMA 295 (2006) 1003–1010.
[25] K. Moazzami, A. Roohi, B. Moazzami, Granulocyte colony stimulating factor therapy for acute myocardial infarction, Cochrane Database Syst. Rev. 5 (2013), CD008844.
[26] E. Deindl, M.M. Zaruba, S. Brunner, et al., G-CSF administration after myocardial infarction in mice attenuates late ischemic cardiomyopathy by enhanced arteriogenesis, FASEB J. 20 (2006) 956–958.
[27] D. Orlic, J. Kajstura, S. Chimenti, et al., Mobilized bone marrow cells repair the infarcted heart, improving function and survival, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 10344–10349.
[28] M.E. Cox, J. Rowell, L. Corsino, J.B. Green, Dipeptidyl peptidase-4 inhibitors in the management of type 2 diabetes: safety, tolerability, and efficacy, Drug Healthc. Patient Saf. 2 (2010) 7–19.
[29] S.J. Reinstadler, H.J. Feistritzer, G. Klug, et al., High-sensitivity troponin T for prediction of left ventricular function and infarct size one year following ST-elevation myocardial infarction, Int. J. Cardiol. 202 (2015) 188–193.